

Frequently Asked Questions:
Biological Safety Guidance for Research with Risk Group 3 Influenza Viruses:
Human H2N2, 1918 H1N1, and HPAI
H5N1 (wild type and mammalian-transmissible by respiratory droplets)

The *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines)* were amended in 2009 and 2013 to provide additional biosafety guidance for research involving Risk Group (RG) 3 influenza viruses, including human H2N2 (1957-1968), 1918 H1N1, and highly pathogenic avian influenza (HPAI) H5N1 viruses, both wild type and genetically modified strains that are mammalian-transmissible by respiratory droplet (mammalian-transmissible HPAI H5N1). The following frequently asked questions are intended to assist investigators and Institutional Biosafety Committees (IBCs) in making decisions about containment and biosafety practices for research with these viruses or their genetic elements.

1. What are the Risk Group classifications for influenza viruses?

In [Appendix B](#) of the *NIH Guidelines*, influenza viruses are classified as either

“Risk Group 2:

Orthomyxoviruses

--Influenza viruses types A, B, and C (except those listed in [Appendix B-III-D](#), *Risk Group 3 (RG3) - Viruses and Prions*).”

RG2 viruses include those that are associated with human disease which is rarely serious (e.g., viruses for which immunity exists in the human population) and for which preventive or therapeutic interventions are often available.

or

“Risk Group 3:

Orthomyxoviruses

--Influenza viruses 1918-1919 H1N1 (1918 H1N1), human H2N2 (1957-1968), and highly pathogenic avian influenza H5N1 strains within the Goose/Guangdong/96-like H5 lineage (HPAI H5N1).”

The HPAI H5N1 viruses include those that are genetically modified to be mammalian-transmissible by respiratory droplets. RG3 viruses are associated with serious or lethal human disease (e.g., viruses with pandemic potential due to the lack of immunity in the human population) for which preventive or therapeutic interventions may be available.

2. Since human H2N2, 1918 H1N1, and HPAI H5N1 influenza viruses are classified as RG3 agents, should research with these viruses be conducted at Biosafety Level 3 (BL3)?

Research should be conducted at BL3 with the enhancements specified in [Appendix G-II-C-5](#). For RG3 influenza viruses, the additional enhancements to containment and practices including occupational health practices reflect the ability of these viruses to spread by respiratory droplets and potentially to cause a pandemic. After the publication in 2012 of two studies describing recombinant laboratory strains of HPAI H5N1 viruses that could transmit by respiratory droplets among ferrets, the *NIH Guidelines* were amended again to delineate additional BL3 enhancements for containment, practices, and occupational health requirements for research involving mammalian-transmissible HPAI H5N1 strains.

3. What enhancements to BL3 containment are specified in the *NIH Guidelines* for research with RG3 influenza viruses?

In addition to standard BL3 facilities and practices ([Appendix G-II-C](#)), the following are some of the additional personal protective equipment (PPE) and practices that shall be used (for the complete list, please see [Appendix G-II-C-5](#)):

- Powered Air-purifying Respirators (PAPR).
- A change of street clothes to protective suit (e.g., wrap-back disposable gown, olefin protective suit).
- Double gloves.
- Appropriate shoe coverings (e.g., double disposable shoe coverings, single disposable shoe coverings if worn with footwear dedicated to BL3 enhanced laboratory use, or impervious boots or shoes of rubber or other suitable material that can be decontaminated).
- Showers prior to exiting the laboratory should be considered depending on risk assessment of research activities. Showers are required for research with mammalian-transmissible HPAI H5N1.

Proper training and periodic assessments of laboratory workers, as well as the reporting of all spills and accidents is required. [Appendix G-II-C-5-b](#) describes containment for animal research.

4. What are the additional containment enhancements for research with HPAI H5N1 influenza viruses that are transmissible among mammals by respiratory droplets?

In addition to the standard and enhanced RG3 influenza virus research BL3 containment ([Appendix G-II-C](#)), the following additional facility air and waste handling requirements shall be followed:

[Appendix G-II-C-2-n](#) - All wastes from laboratories and animal rooms are appropriately decontaminated before disposal. For research involving mammalian-transmissible HPAI H5N1 virus, liquid effluents should be chemically disinfected or heat-treated, or collected

and processed in a central effluent decontamination system. Decontamination of shower and toilet effluents is not required, provided appropriate practices and procedures are in place for primary containment of mammalian-transmissible HPAI H5N1 virus. Animal tissues, carcasses, and bedding originating from the animal room must be decontaminated by an effective and validated method (e.g., use of an autoclave) preferably before leaving the containment barrier. If waste must be transported, special practices should be developed for transport of infectious materials to designated alternate location(s) within the facility.

Appendix G-II-C-4-i - For research with mammalian-transmissible HPAI H5N1 virus, exhaust air must be HEPA filtered and there must be sealed ductwork from the containment barrier to the filter. In addition, the air handling system shall be designed such that under failure conditions, the airflow will not be reversed and periodic verification, with annual verification of the HEPA filters, shall be performed. Backup power shall be available for critical controls and instrumentation necessary to maintain containment.

5. What are the additional practices and training enhancements for research with HPAI H5N1 influenza viruses that are transmissible among mammals by respiratory droplets?

In addition to the practices and PPE to be used for research with the other RG3 influenza viruses, the following must be used:

- Showers prior to exiting the laboratory are required for all research, including care of animals infected with mammalian-transmissible HPAI H5N1 virus.
- Prior to exiting containment, PPE shall be sprayed or wiped down with a disinfectant that has activity against influenza viruses.
- In order to promote adherence to proper practices, and reporting of any loss of containment or exposures, at least two individuals should be in the laboratory at all times when research with mammalian-transmissible HPAI H5N1 virus involves experimental procedures with animals or sharps, or when procedures are being conducted whereby the generation of aerosols is reasonably anticipated. In addition, in order to ensure proper removal of personal protective equipment this should be observed, either directly or by video monitoring that is reviewed within 24 hours.
- Laboratory workers shall be required to sign a document acknowledging their understanding of and intent to adhere to biosafety, biosecurity, and occupational health requirements. This document shall include a statement that the laboratory worker agrees to report any exposures or accidents, including those by other individuals in the lab.

6. What are the occupational health requirements for research with RG3 influenza viruses?

A detailed occupational health plan must be developed before working with these viruses. The requirements for the plan are described in **Appendix G-II-C-5** and include the following:

- Laboratory workers are provided medical cards with information regarding the viruses and a 24 hour contact number for the principal investigator (PI) and a designated occupational health provider.
- Annual vaccination against seasonal influenza should be given.
- Virus specific vaccination should be offered if available. H5N1 vaccines that are not yet approved by the FDA (i.e., vaccines being evaluated as investigational new drugs) may be considered for use after consultation with OBA.
- Reporting of respiratory symptoms and/or fever and active monitoring for influenza-like-illness is required for working with mammalian-transmissible H5N1.
- In the event of a potential exposure or development of an influenza-like-illness in a laboratory worker, there shall be 24 hour access to a medical facility prepared to implement appropriate respiratory isolation, provide antiviral agents, and test to determine whether the infection is due to a recombinant or synthetic influenza virus.
- Baseline serum samples should be collected and stored for research with RG3 agents and this is required for work with mammalian-transmissible H5N1 viruses. Also for work with HPAI H5N1, if a HPAI H5N1 vaccination is available and administered a postvaccination serum sample should be collected, assessed in consultation with appropriate infectious disease and occupational health experts, and stored in accordance with institutional policy.

7. What should be done in the event of a potential or known exposure to a RG3 influenza virus?

In advance of initiating research with RG3 influenza viruses, the PI should develop a plan for a risk assessment to be conducted by the PI and health and biosafety officials to determine the appropriate response, including the extent of isolation of the exposed worker, the need for initiation of antiviral treatment, and notification of appropriate public health authorities. In the case of a known exposure with a high risk for infection (e.g., involving the upper or lower respiratory tract or mucous membranes) to wild-type HPAI H5N1, laboratory workers should be prepared to self-isolate until infection can be ruled out. For known high risk exposures to human H2N2, 1918 H1N1, or mammalian-transmissible HPAI H5N1, laboratory workers must be isolated in a predetermined facility (i.e. not at home) until infection can be ruled out by testing. Antiviral agents may not be provided in advance (i.e. no home supplies) but only after medical evaluation for research with 1918 H1N1 and mammalian-transmissible HPAI H5N1 viruses.

8. Are there additional considerations if research involves RG3 influenza viruses that are resistant to antiviral agents?

The availability of antiviral drugs as a preventive and therapeutic measure is an important safeguard for experiments with 1918 H1N1, HPAI H5N1, and human H2N2. [Section III-D-7-d](#), Antiviral Susceptibility and Containment, states that if an influenza virus containing genes from one of these viruses is resistant to both classes of current antiviral agents, adamantanes and neuraminidase inhibitors, higher containment may be required based on the risk assessment.

Experiments that are designed to create resistance to neuraminidase inhibitors or other effective antiviral agents (including investigational antiviral agents being developed for influenza) would be subject to [Section III-A-1](#) (Major Actions) and require RAC review and NIH Director approval. If the agent is a Select Agent, the NIH will defer to the appropriate Federal agency (HHS or USDA Select Agent Divisions) on such experiments. For experiments that are not intended to generate resistance but which involve manipulation of the genes that influence sensitivity to antiviral agents, continued susceptibility to these agents shall be reconfirmed ([Appendix G-II-C-5-a-\(5\)](#)). If susceptibility to neuraminidase inhibitors or other effective antiviral agents is lost as a result of genetic modification or serial passage of a **mammalian-transmissible HPAI H5N1 virus**, then any research with this antiviral agent-resistant virus shall be stopped and research shall proceed only after review by the NIH or the appropriate Federal regulatory agency.

9. Are there certain experiments involving genes and/or segments from RG3 influenza viruses, which can be conducted at lower levels of containment?

Experiments with influenza viruses containing genes or segments from 1918 H1N1, human H2N2, HPAI H5N1 including strains that are transmissible among mammals by respiratory droplets shall be conducted at BL3 enhanced containment. However, an IBC may consider lower containment in certain cases:

- **Human H2N2:** Experiments with the H2 HA gene in cold-adapted, live attenuated vaccine strains (e.g., A/Ann Arbor/6/60 H2N2) may be conducted at BL2 containment provided segments with mutations conferring temperature sensitivity and attenuation are not altered in the recombinant or synthetic virus. Experiments with Risk Group 2 influenza viruses containing genes from human H2N2 other than the HA gene can be worked on at BL2 ([Section III-D-7-a](#)).
- **HPAI H5N1:** Experiments involving influenza viruses containing a minority of genes and/or segments from a non-mammalian transmissible HPAI H5N1 influenza virus shall be conducted at BL3 enhanced unless a risk assessment performed by the IBC determines that they can be conducted safely at BL 2 based on results from at least two animal models (e.g., ferret, mouse, Syrian golden hamster, cotton rat, non-human primates) that demonstrate that the resulting influenza virus shows reduced replication and virulence compared to the parental RG3 virus at relevant doses. This should be determined by measuring biological indices appropriate for the specific animal model (e.g., severe weight loss, elevated temperature, mortality or neurological symptoms). IBCs may request assistance from OBA to provide consultation with the RAC and influenza virus experts. For certain HPAI H5N1 viruses, [USDA/APHIS](#) regulations and decisions on lowering containment for select agents also apply. ([Section III-D-7-b](#)).
- **1918 H1N1:** Experiments involving influenza viruses containing any gene or segment from 1918 H1N1 must always be conducted at BL3 enhanced containment ([Section III-D-7-c](#)).

10. Are there are particular terms and conditions of award that NIH places on work with RG3 influenza viruses?

As stated in [Section I-D](#) of the *NIH Guidelines* and reiterated in the [NIH Grants Policy Manual](#), as a condition for NIH funding of recombinant DNA research, institutions shall ensure that such research conducted at or sponsored by the institution, irrespective of the source of funding, shall comply with the *NIH Guidelines*.

NIH Institutes and Centers may have their own terms and conditions of grant award in addition to the standards articulated by the *NIH Guidelines* and *Biosafety in Microbiological and Biomedical Laboratories* (BMBL). You should consult with your NIH program officer for information on any such terms and conditions.

11. Where can I find out more information on conducting a risk assessment, containment, and practices for research with RG3 influenza strains?

Information may be found in the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules*:

[Section III-D-7](#) - Experiments Involving Influenza Viruses and [Section G-II-C-5](#) - Biosafety Level 3 Enhanced for Research Involving Risk Group 3 Influenza Viruses.

Further guidance on agents may be obtained through:

- National Institutes of Health, Office of Biotechnology Activities

Phone: 301-496-9838

Email: oba-osp@od.nih.gov

Web Site: <http://osp.od.nih.gov/office-biotechnology-activities>

- Centers for Disease Control and Prevention, Select Agent Program

Phone: 404-718-2000

Email: lrsat@cdc.gov

Web Site: <http://www.cdc.gov/od/sap/>

- United States Department of Agriculture, Animal and Plant Health Inspection Service, Select Agent Program

Phone: 301-734-5960

Email: Agricultural.Select.Agent.Program@aphis.usda.gov

Web Site: http://www.aphis.usda.gov/programs/ag_selectagent/